

## PIXELGEN

#### TECHNOLOGIES

# 3D SPATIAL PROTEOMICS BY MOLECULAR PIXELATION APPLIED ON SINGLE CELLS

Filip Karlsson<sup>1</sup>, Tomasz Kallas<sup>1</sup>, Divya Thiagarajan<sup>1</sup>, Max Karlsson<sup>1</sup>, Maud Schweitzer<sup>1</sup>, Jose Fernandez Navarro<sup>1</sup>, Louise Leijonancker<sup>1</sup>, Sylvain Geny<sup>1</sup>, Erik Pettersson<sup>1</sup>, Jan Rhomberg-Kauert<sup>1</sup>, Marcela Gonzalez Granillo<sup>1</sup>, Jessica Bunz<sup>1</sup>, Johan Dahlberg<sup>1</sup>, Michele Simonetti<sup>1</sup>, Prajakta Sathe<sup>1</sup>, Petter Brodin<sup>3,4</sup>, Alvaro Martinez Barrio<sup>1</sup>, Simon Fredriksson<sup>1,2</sup>, AnnaLotta Schiller Vestergren<sup>1</sup>, Annika Branting<sup>1</sup>, Steve Glavas<sup>1</sup>

<sup>1</sup>Pixelgen Technologies AB, Stockholm, Sweden <sup>2</sup>Royal Institute of Technology, Department of Protein Science, Stockholm, Sweden <sup>3</sup> Dept. of Women's and Children's Health, Karolinska Institutet, 17165, Solna, Sweden <sup>4</sup> Dept. of Immunology and Inflammation, Imperial College London, W12 ONN, London, UK

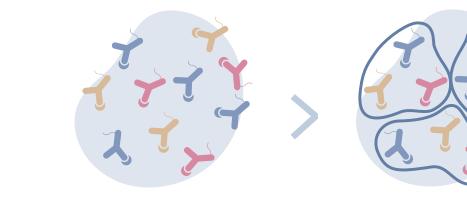
## GO BEYOND WHAT YOU CAN DO TODAY -UNLOCK SPATIAL SURFACE PROTEOME

The cell surface proteome is spatially dynamic and changes with the state of the cell, which in turn determines its activity in health and disease. Understanding differential gene regulation, post-transcriptional changes and variations in protein translation alone is insufficient to fully comprehend what causes the onset of disease, progression and response to treatment.

#### **Molecular Pixelation (MPX) Workflow**

**Pixelation A** 

**Antibody Binding** 



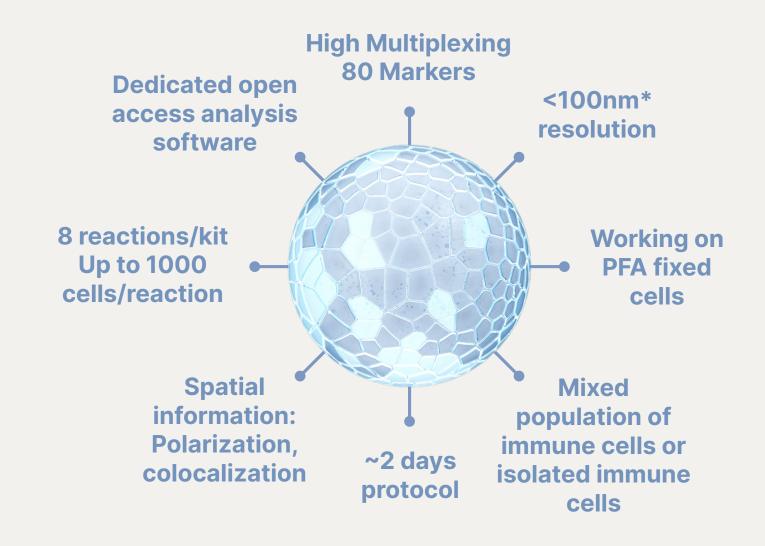
Molecular Pixelation A Cells in solution bound by oligo-linked antibodies -

preparation

Molecular Pixelation B creating spatial correl

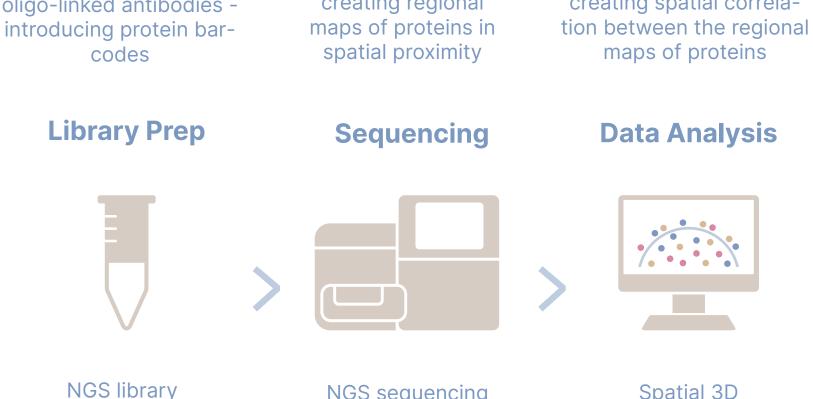
**Pixelation B** 





**MOLECULAR PIXELATION** (MPX) ENABLES YOU TO VISUALIZE CHANGES IN THE SPATIAL ARCHITECTURE OF MEMBRANE PROTEINS ON SINGLE CELLS

- Opening up for detailed analysis of vital processes of the immune system, such as cell-cell communication and mobility
- Detecting marker abundance with polarization and colocalization patterns in 3D space.





\*nanometer size as measured by scanning electron microscopy.

### **APPLICATIONS**

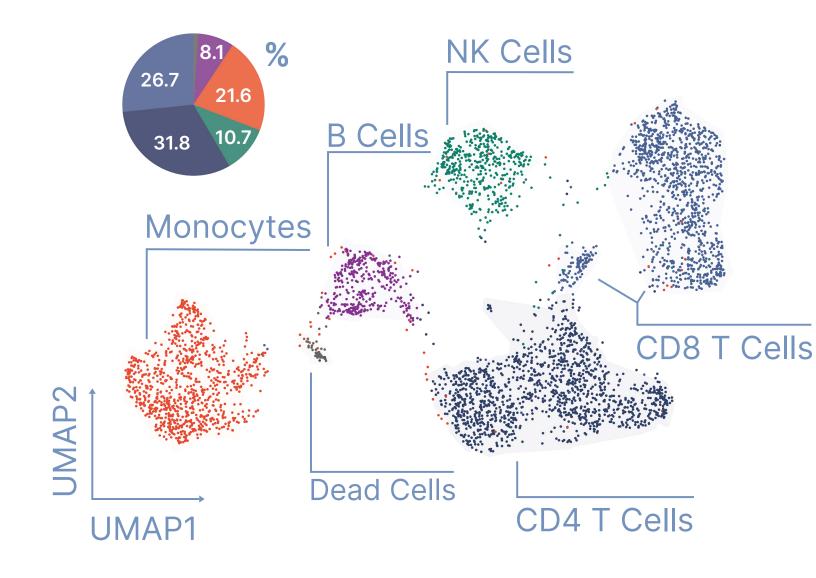
**Cell-cell Communication** 

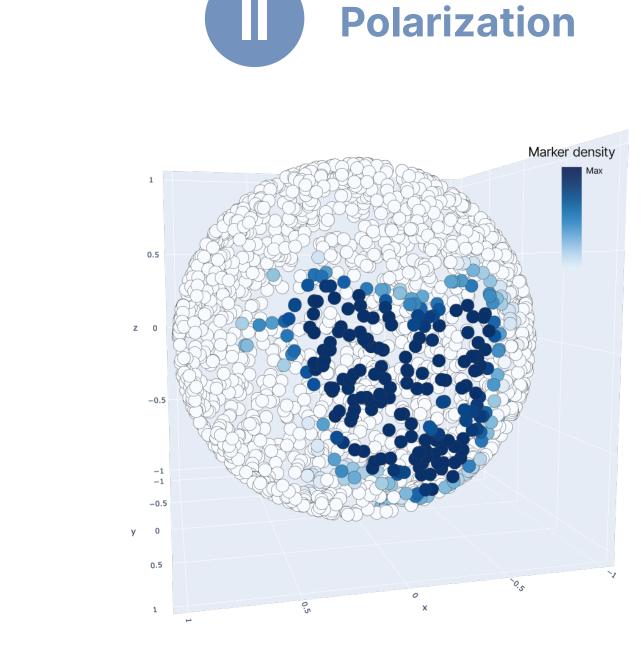
**Cell Migration** 

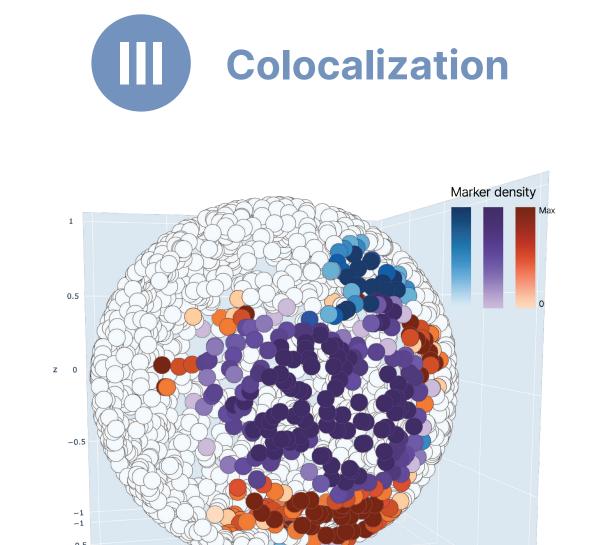
**Drug Response** 

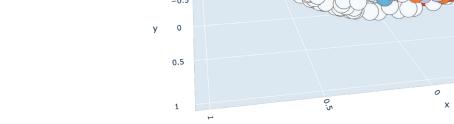
# HALLMARKS OF MOLECULAR PIXELATION



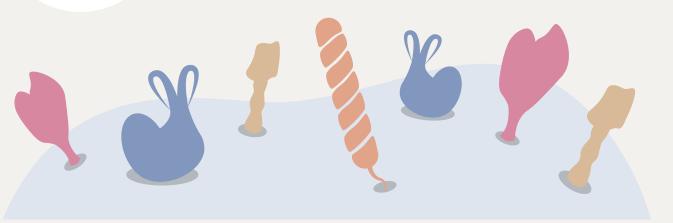












### **COLOCALIZATION OF PROTEIN PAIRS IN** UROPODS OF CHEMOTACTIC T CELLS

Uropod formation is an essential mechanism for cytotoxic T cells to infiltrate tumors, which correlates to immune checkpoint inhibition efficacy and overall cancer survival.

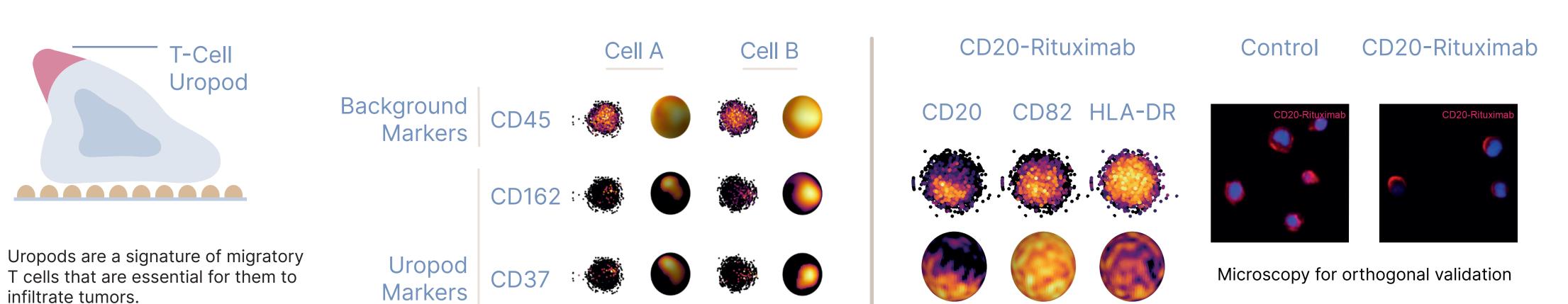
The detection of uropod formation in migrating cells has been extremely hard to study in detail before with existing technologies. With MPX this is now possible. The high multiplexing ability of the method and the graph data generated enables discovery of new colocalization patterns, or the opposite, segregation of proteins in the generated data.

#### POLARIZATION OF CD20 BY **RITUXIMAB**

Therapeutic antibody Rituximab clusters CD20 on B lymphoma cells with represented polarity score. Orthogonal fluorescent microscopy shows the spatial distribution of the target proteins upon stimulation.

**Molecular Pixelation** is a novel addition to the single cell analysis community to to reveal new insights into cellular life.

Our pioneering Molecular Pixelation technology and its dedicated open source software enables simultaneous detection of 80, human immune cell surface markers in parallel. This allows for visualization of receptor abundance, polarization and colocalization of thousands of cells in 3D, at nanoscale resolution.

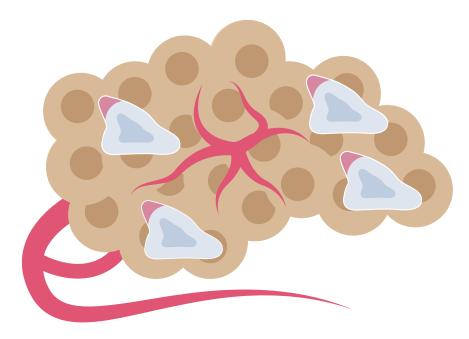


**DEEP PHENOTYPING OF CELLS** 

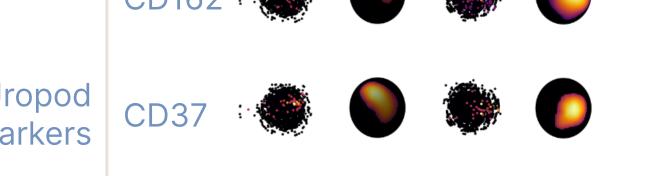
HIGH MULTIPLEX, THROUGHPUT

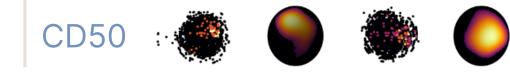


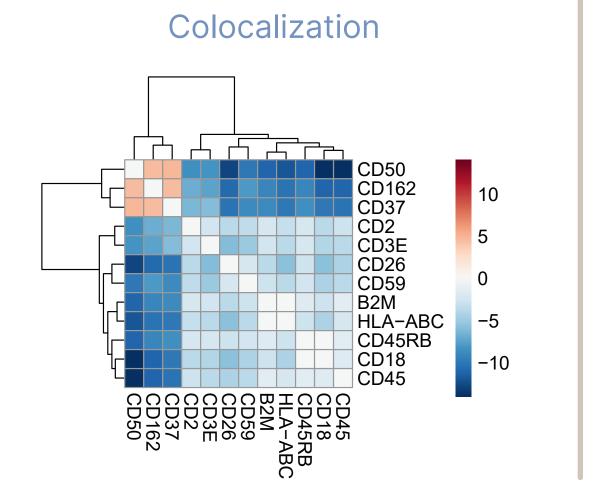
T cells that are essential for them to infiltrate tumors.



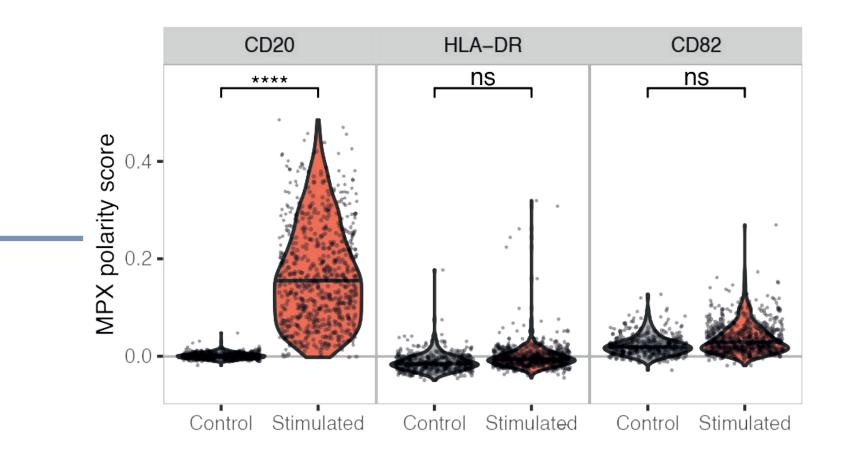
MPX was used to detect uropod formation, providing important insights into T-cell motility, which could help the development of immune therapies.







**Polarity Score** measures the level of clustering of each target protein for each single cell.



**AND RESOLUTION** 

SINGLE CELL SPECIFICITY WITHOUT COMPARTMENTALIZATION

#### **NO SPECIAL EQUIPMENT NEEDED**

To find out more, scan this QR code

